

**Amendments to the Specification**

**Please replace the Section starting on page 3, 'BRIEF DESCRIPTION OF THE DRAWINGS', with the following amended Section:**

**BRIEF DESCRIPTION OF THE DRAWINGS**

Fig. 1 illustrates the products of (B) protein expression and (A) purification, (C) FACs analysis, (D) bactericidal assay, (E) western blot, and (F) ELISA assay of the predicted ORF 919 as cloned and expressed in *E. coli*.

Fig. 2 illustrates the products of (A) protein expression and purification, (B) western blot, (C) FACs analysis, (D) bactericidal assay, and (E) ELISA assay of the predicted ORF 279 as cloned and expressed in *E. coli*.

Fig. 3 illustrates the products of (A) protein expression and purification, (B) western blot, (C) FACs analysis, (D) bactericidal assay, and (E) ELISA assay of the predicted ORF 576-1 as cloned and expressed in *E. coli*.

Fig. 4 illustrates the products of (A) protein expression and purification, (B) western blot, (C) FACs analysis, (D) bactericidal assay, and (E) ELISA assay of the predicted ORF 519-1 as cloned and expressed in *E. coli*.

Fig. 5 illustrates the products of (A) protein expression and purification, (B) western blot, (C) FACs analysis, (D) bactericidal assay, and (E) ELISA assay of the predicted ORF 121-1 as cloned and expressed in *E. coli*.

Fig. 6 illustrates the products of (A) protein expression and purification, (B) western blot, (C) FACs analysis, (D) bactericidal assay, and (E) ELISA assay of the predicted ORF 128-1 as cloned and expressed in *E. coli*.

Fig. 7 illustrates the products of (A) protein expression and purification, (B) western blot, (C) FACs analysis, (D) bactericidal assay, and (E) ELISA assay of the predicted ORF 206 as cloned and expressed in *E. coli*.

Fig. 8 illustrates the products of (A) protein expression and purification, (B) FACs analysis, (C) bactericidal assay, and (D) ELISA assay of the predicted ORF 287 as cloned and expressed in *E. coli*.

Fig. 9 illustrates the products of (A) protein expression and purification, (B) western blot, (C) FACs analysis, (D) bactericidal assay, and (E) ELISA assay of the predicted ORF 406 as cloned and expressed in *E. coli*.

Fig. 10 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 919 as cloned and expressed in *E. coli*.

Fig. 11 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 279 as cloned and expressed in *E. coli*.

Fig. 12 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 576-1 as cloned and expressed in *E. coli*.

Fig. 13 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 519-1 as cloned and expressed in *E. coli*.

Fig. 14 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 121-1 as cloned and expressed in *E. coli*.

Fig. 15 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 128-1 as cloned and expressed in *E. coli*.

Fig. 16 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 206 as cloned and expressed in *E. coli*.

Fig. 17 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 287 as cloned and expressed in *E. coli*.

Fig. 18 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 406 as cloned and expressed in *E. coli*.

Fig. 19A-C shows an alignment comparison of amino acid sequences for ORF 225 for several strains of *Neisseria*. Dark shading indicates regions of homology, and gray shading indicates the conservation of amino acids with similar characteristics. The Figure demonstrates a high degree of conservation among the various strains, further confirming its utility as an antigen for both vaccines and diagnostics. The sequences in the Figure have the following SEQ ID NOs: FA1090 SEQ ID 3115; Z2491 SEQ ID 3116; ZO01 225 SEQ ID 3117; ZO02 225 SEQ ID 3118; ZO03 225 SEQ ID 3119; ZO04 225 SEQ ID 3120; ZO05 225 SEQ ID 3121; ZO06 225 SEQ ID 3122; ZO07 225 SEQ ID 3123; ZO08 225 SEQ ID 3124; ZO09 225 SEQ ID 3125; ZO10 225 SEQ ID 3126; ZO11 225 SEQ ID 3127; ZO12 225 SEQ ID 3128; ZO13 225 SEQ ID 3129; ZO14 225 SEQ ID 3130; ZO15 225 <SEQ ID 3131; ZO16 225 SEQ ID 3132; ZO17 225 SEQ ID 3133; ZO18 225 SEQ ID 3134; ZO19 225 SEQ ID 3135; ZO20 225 SEQ ID 3136; ZO21 225 SEQ ID 3137; ZO22 225 SEQ ID 3138; ZO23 225 SEQ ID 3139; ZO24 225 SEQ ID 3140; ZO25 225 SEQ ID 3141; ZO26 225 SEQ ID 3142; ZO27 225 SEQ ID 3143; ZO28 225 SEQ ID 3144; ZO29 225 SEQ ID 3145; ZO32 225 SEQ ID 3146; ZO33 225 SEQ ID 3147; and ZO96 225 SEQ ID 3148.

Fig. 20A-B shows an alignment comparison of amino acid sequences for ORF 235 for several strains of *Neisseria*. Dark shading indicates regions of homology, and gray shading indicates the conservation of amino acids with similar characteristics. The Figure demonstrates a high degree of conservation among the various strains, further confirming its utility as an antigen for both vaccines and diagnostics. The sequences in the Figure have the following SEQ ID NOs: FA1090 SEQ ID 3149; GNMZQ01 SEQ ID 3150; GNMZQ02 SEQ ID 3151; GNMZQ03 SEQ ID 3152; GNMZQ04 SEQ ID 3153; GNMZQ05 SEQ ID 3154; GNMZQ07 SEQ ID 3155; GNMZQ08 SEQ ID 3156; GNMZQ09 SEQ ID 3157; GNMZQ10 SEQ ID 3158; GNMZQ11 SEQ ID 3159;

GNMZQ13 SEQ ID 3160; GNMZQ14 SEQ ID 3161; GNMZQ15 SEQ ID 3162; GNMZQ16 SEQ ID 3163; GNMZQ17 SEQ ID 3164; GNMZQ18 SEQ ID 3165; GNMZQ19 SEQ ID 3166; GNMZQ21 SEQ ID 3166; GNMZQ22 SEQ ID 3167; GNMZQ23 SEQ ID 3168; GNMZQ24 SEQ ID 3169; GNMZQ25 SEQ ID 3170; GNMZQ26 SEQ ID 3171; GNMZQ27 SEQ ID 3172; GNMZQ28 SEQ ID 3173; GNMZQ29 SEQ ID 3174; GNMZQ31 SEQ ID 3175; GNMZQ32 SEQ ID 3176; GNMZQ33 SEQ ID 3177; and Z2491 SEQ ID 3178.

Fig. 21A-B shows an alignment comparison of amino acid sequences for ORF 287 for several strains of *Neisseria*. Dark shading indicates regions of homology, and gray shading indicates the conservation of amino acids with similar characteristics. The Figure demonstrates a high degree of conservation among the various strains, further confirming its utility as an antigen for both vaccines and diagnostics. The sequences in the Figure have the following SEQ ID NOs: 287\_14 SEQ ID 3179; 287\_2 SEQ ID 3180; 287\_21 SEQ ID 3181; 287\_9 SEQ ID 3182; FA1090 SEQ ID 3183; and Z2491 SEQ ID 3184.

Fig. 22A-B shows an alignment comparison of amino acid sequences for ORF 519 for several strains of *Neisseria*. Dark shading indicates regions of homology, and gray shading indicates the conservation of amino acids with similar characteristics. The Figure demonstrates a high degree of conservation among the various strains, further confirming its utility as an antigen for both vaccines and diagnostics. The sequences in the Figure have the following SEQ ID NOs: FA1090\_519 SEQ ID 3185; Z2491\_519 SEQ ID 3186; ZV01\_519 SEQ ID 3187; ZV02\_519 SEQ ID 3188; ZV03\_519 SEQ ID 3189; ZV04\_519 SEQ ID 3190; ZV05\_519 SEQ ID 3191; ZV06\_519ASS SEQ ID 3192; ZV07\_519 SEQ ID 3193; ZV11\_519 SEQ ID 3194; ZV12\_519 SEQ ID 3195; ZV18\_519 SEQ ID 3196; ZV19\_519 SEQ ID 3197; ZV20\_519ASS SEQ ID 3198; ZV21\_519ASS SEQ ID 3199; ZV22\_519ASS SEQ ID 3200; ZV26\_519 SEQ ID 3201; ZV27\_519 SEQ ID 3202; ZV28\_519 SEQ ID 3203; ZV29\_519ASS SEQ ID 3204; ZV32\_519 SEQ ID 3205; and ZV96\_519 SEQ ID 3206.

Fig. 23A-D shows an alignment comparison of amino acid sequences for ORF 919 for several strains of *Neisseria*. Dark shading indicates regions of homology, and gray shading indicates

the conservation of amino acids with similar characteristics. The Figure demonstrates a high degree of conservation among the various strains, further confirming its utility as an antigen for both vaccines and diagnostics. The sequences in the Figure have the following SEQ ID NOs: FA1090 SEQ ID 3207; Z2491 <SEQ ID 3208; ZM01 SEQ ID 3209; ZM02 SEQ ID 3210; ZM03 SEQ ID 3211; ZM04 SEQ ID 3212; ZM05 SEQ ID 3213; ZM06 SEQ ID 3214; ZM07 SEQ ID 3215; ZM08N SEQ ID 3216; ZM09 SEQ ID 3217; ZM10 SEQ ID 3218; ZM11ASBC SEQ ID 3219; ZM12 SEQ ID 3220; ZM13 SEQ ID 3221; ZM14 SEQ ID 3222; ZM15 SEQ ID 3223; ZM16 SEQ ID 3224; ZM17 SEQ ID 3225; ZM18 SEQ ID 3226; ZM19 SEQ ID 3227; ZM20 SEQ ID 3228; ZM21 SEQ ID 3229; ZM22 SEQ ID 3230; ZM23ASBC SEQ ID 3231; ZM24 SEQ ID 3232; ZM25 SEQ ID 3233; ZM26 SEQ ID 3234; ZM27BC SEQ ID 3235; ZM28 SEQ ID 3236; ZM29ASBC SEQ ID 3237; ZM31ASBC SEQ ID 3238; ZM32ASBC SEQ ID 3239; ZM33ASBC SEQ ID 3240; ZM96 SEQ ID 3241.

**Please replace the paragraph spanning pages 15 and 16, with the following amended paragraph:**

After inserting the DNA sequence encoding the protein into the transfer vector, the vector and the wild type viral genome are transfected into an insect host cell where the vector and viral genome are allowed to recombine. The packaged recombinant virus is expressed and recombinant plaques are identified and purified. Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form from, *inter alia*, Invitrogen, San Diego CA ("~~MaxBac~~" "MAXBAC(TM)" kit). These techniques are generally known to those skilled in the art and fully described in Summers and Smith, *Texas Agricultural Experiment Station Bulletin No. 1555* (1987) (hereinafter "Summers and Smith").

**Please replace the paragraph beginning on page 35, line 5, with the following amended paragraph:**

Preferred adjuvants to enhance effectiveness of the composition include, but are not limited to: (1) aluminum salts (alum), such as aluminum hydroxide, aluminum phosphate, aluminum sulfate, etc;

(2) oil-in-water emulsion formulations (with or without other specific immunostimulating agents such as muramyl peptides (see below) or bacterial cell wall components), such as for example (a) MF59 (PCT Publ. No. WO 90/14837), containing 5% Squalene, 0.5% ~~Tween-80~~ TWEEN 80(TM), and 0.5% ~~Span-85~~ SPAN 85(TM) (optionally containing various amounts of MTP-PE (see below), although not required) formulated into submicron particles using a microfluidizer such as Model 110Y microfluidizer (Microfluidics, Newton, MA), (b) SAF, containing 10% Squalene, 0.4% Tween 80, 5% ~~pluronic~~ PLURONIC(TM)-blocked polymer L121, and thr-MDP (see below) either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion, and (c) RIBI(TM) ~~Ribi~~<sup>TM</sup> adjuvant system (RAS), (Ribi Immunochem, Hamilton, MT) containing 2% Squalene, 0.2% ~~Tween-80~~ TWEEN 80(TM), and one or more bacterial cell wall components from the group consisting of monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (~~DETOX(TM)~~ Detox<sup>TM</sup>); (3) saponin adjuvants, such as STIMULON(TM) ~~Stimulon~~<sup>TM</sup> (Cambridge Bioscience, Worcester, MA) may be used or particles generated therefrom such as ISCOMs (immunostimulating complexes); (4) Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (IFA); (5) cytokines, such as interleukins (e.g., IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12, *etc.*), interferons (e.g., gamma interferon), macrophage colony stimulating factor (M-CSF), tumor necrosis factor (TNF), etc; and (6) other substances that act as immunostimulating agents to enhance the effectiveness of the composition. Alum and MF59 are preferred.

**Please replace the paragraph on page 38, line 7, with the following amended paragraph:**

Preferred retroviruses for the construction of retroviral gene therapy vectors include Avian Leukosis Virus, Bovine Leukemia[[,]] Virus, Murine Leukemia Virus, Mink-Cell Focus-Inducing Virus, Murine Sarcoma Virus, Reticuloendotheliosis Virus and Rous Sarcoma Virus. Particularly preferred Murine Leukemia Viruses include 4070A and 1504A (Hartley and Rowe (1976) *J Virol* 19:19-25), Abelson (ATCC No. VR-999), Friend (ATCC No. VR-245), Graffi, Gross (ATCC Nol VR-590), Kirsten, Harvey Sarcoma Virus and Rauscher (ATCC No. VR-998) and Moloney Murine Leukemia Virus (ATCC No. VR-190). Such retroviruses may be obtained

from depositories or collections such as the American Type Culture Collection ("ATCC") in ~~Rockville, Maryland~~ or isolated from known sources using commonly available techniques.

**Please replace the paragraph on page 40, line 4, with the following amended paragraph:**

Also contemplated are alpha virus gene therapy vectors that can be employed in this invention. Preferred alpha virus vectors are Sindbis viruses vectors. Togaviruses, Semliki Forest virus (ATCC VR-67; ATCC VR-1247), Middleberg virus (ATCC VR-370), Ross River virus (ATCC VR-373; ATCC VR-1246), Venezuelan equine encephalitis virus (ATCC VR923; ATCC VR-1250; ATCC VR-1249; ATCC VR-532), and those described in US patents 5,091,309, 5,217,879, and WO92/10578. More particularly, those alpha virus vectors described in U.S. Serial No. 08/405,627, filed March 15, 1995, WO94/21792, WO92/10578, WO95/07994, US 5,091,309 and US 5,217,879 are employable. Such alpha viruses may be obtained from depositories or collections such as the ATCC in ~~Rockville, Maryland~~ or isolated from known sources using commonly available techniques. Preferably, alphavirus vectors with reduced cytotoxicity are used (see USSN 08/679640)

**Please replace the paragraph on page 47, line 18, with the following amended paragraph:**

Synthetic polycationic agents which are useful include, for example, DEAE-dextran, polybrene, LIPOFECTIN(TM), and LIPOFECTAMINE(TM) ~~Lipofectin<sup>®</sup>, and lipofectAMINE<sup>®</sup>~~ are monomers that form polycationic complexes when combined with polynucleotides or polypeptides.

**Please replace the paragraph on page 52, line 22, with the following amended paragraph:**

Sequence comparisons were performed at NCBI (<http://www.ncbi.nlm.nih.gov>) using the algorithms BLAST, BLAST2, BLASTn, BLASTp, tBLASTn, BLASTx, & tBLASTx [eg. see also Altschul *et al.* (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* 25:2289-3402]. Searches were performed against the

following databases: non-redundant GenBank+EMBL+DDBJ+PDB sequences and non-redundant GenBank CDS translations+PDB+SwissProt+SPupdate+PIR sequences.

**Please replace the paragraph on page 53, line 10, with the following amended paragraph:**

Underlined amino acid sequences indicate possible transmembrane domains or leader sequences in the ORFs, as predicted by the PSORT algorithm (<http://www.psort.nibb.ac.jp>). Functional domains were also predicted using the MOTIFS program (GCG Wisconsin & PROSITE).

**Please replace the paragraph on page 53, line 26, with the following amended paragraph:**

*N. meningitidis* strain 2996 was grown to exponential phase in 100 ml of GC medium, harvested by centrifugation, and resuspended in 5 ml buffer (20% Sucrose, 50 mM Tris-HCl, 50 mM EDTA, pH 8). After 10 minutes incubation on ice, the bacteria were lysed by adding 10 ml lysis solution (50 mM NaCl, 1% Na-SARKOSYL(TM) ~~Sarkosyl~~, 50 µg/ml Proteinase K), and the suspension was incubated at 37°C for 2 hours. Two phenol extractions (equilibrated to pH 8) and one  $\text{ChCl}_3$ /isoamylalcohol (24:1) extraction were performed. DNA was precipitated by addition of 0.3M sodium acetate and 2 volumes ethanol, and was collected by centrifugation. The pellet was washed once with 70%(v/v) ethanol and redissolved in 4.0ml TE buffer (10 mM Tris-HCl, 1mM EDTA, pH 8.0). The DNA concentration was measured by reading the OD at 260 nm.

**Please replace the paragraph on page 55, line 20, with the following amended paragraph:**

Oligos were synthesized using a Perkin Elmer 394 DNA/RNA SYNTHESIZER(TM) ~~Synthesizer~~, eluted from the columns in 2.0 ml  $\text{NH}_4\text{OH}$ , and deprotected by 5 hours incubation at 56°C. The oligos were precipitated by addition of 0.3M Na-Acetate and 2 volumes ethanol. The samples were centrifuged and the pellets resuspended in either 100µl or 1.0ml of water. The  $\text{OD}_{260}$  was determined using a Perkin Elmer LAMBDA BIO(TM) ~~Lambda Bio~~ spectrophotometer and the concentration adjusted to 2-10pmol/µl.



**Please replace the paragraph spanning pages 55 and 56, with the following amended paragraph:**

The standard PCR protocol was as follows: 50-200 ng of genomic DNA was used as a template in the presence of 20-40  $\mu$ M of each oligonucleotide primer, 400-800  $\mu$ M dNTPs solution, 1x PCR buffer (including 1.5 mM  $MgCl_2$ ), 2.5 units *TaqI* DNA polymerase (using Perkin-Elmer AMPLITAQ(TM) ~~AmpliTaQ~~, GIBCO Platinum, Pwo DNA polymerase, or Tahara Shuzo Taq polymerase). In some cases, PCR was optimised by the addition of 10 $\mu$ l of DMSO or 50 $\mu$ l of 2M Betaine.

**Please replace the paragraph beginning on page 60, line 4, with the following amended paragraph:**

For some ORFs, a single colony was grown overnight at 37°C on LB+Amp agar plate. The bacteria were inoculated into 20 ml of LB+Amp liquid ~~culture~~ culture in a water bath shaker and grown overnight. Bacteria were diluted 1:30 into 600 ml of fresh medium and allowed to grow at the optimal temperature (20-37°C) to OD<sub>550</sub> 0.8-1. Protein expression was induced with 0.2mM IPTG followed by three hours incubation. The culture was centrifuged at 8000 rpm at 4°C. The supernatant was discarded and the bacterial pellet was resuspended in 7.5 ml cold PBS. The cells were disrupted by sonication on ice for 30 sec at 40W using a Branson sonifier B-15, frozen and thawed two times and centrifuged again. The supernatant was collected and mixed with 150 $\mu$ l GLUTATHIONE-SEPHAROSE 4B(TM) ~~Glutathione-Sepharese 4B~~ resin (Pharmacia) (previously washed with PBS) and incubated at room temperature for 30 minutes. The sample was centrifuged at 700g for 5 minutes at 4C. The resin was washed twice with 10 ml cold PBS for 10 minutes, resuspended in 1ml cold PBS, and loaded on a disposable column. The resin was washed twice with 2ml cold PBS until the flow-through reached OD<sub>280</sub> of 0.02-0.06. The GST-fusion protein was eluted by addition of 700 $\mu$ l cold Glutathione elution buffer (10mM reduced glutathione, 50mM Tris-HCl) and fractions collected until the OD<sub>280</sub> was 0.1. 21 $\mu$ l of each fraction were loaded on a 12% SDS gel using either Biorad SDS-PAGE Molecular weight standard broad range (M1) (200, 116.25, 97.4, 66.2, 45, 31, 21.5, 14.4, 6.5 kDa) or Amersham Rainbow Marker (M'') (220, 66, 46,

30, 21.5, 14.3 kDa) as standards. As the MW of GST is 26kDa, this value must be added to the MW of each GST-fusion protein.

**Please replace the paragraph beginning on page 61, line 1, with the following amended paragraph:**

The bacterial pellet was resuspended in 7.5ml cold PBS. Cells were disrupted by sonication on ice for 30 sec at 40W using a Branson sonifier 450 and centrifuged at 13 000xg for 30 min at 4°C. The supernatant was collected and mixed with 150µl GLUTATHIONE-SEPHAROSE 4B(TM) ~~Glutathione-Sepharose 4B~~ resin (Pharmacia), previously equilibrated with PBS, and incubated at room temperature with gentle agitation for 30 min. The batch-wise preparation was centrifuged at 700xg for 5 min at 4°C and the supernatant discarded. The resin was washed twice (batchwise) with 10ml cold PBS for 10 min, resuspended in 1ml cold PBS, and loaded onto a disposable column. The resin continued to be washed twice with cold PBS, until the OD<sub>280nm</sub> of the flow-through reached 0.02-0.01. The GST-fusion protein was eluted by addition of 700µl cold glutathione elution buffer (10mM reduced glutathione, 50mM Tris-HCl pH 8.0) and fractions collected, until the OD<sub>280nm</sub> of the eluate indicated all the recombinant protein was obtained. 20µl aliquots of each elution fraction were analyzed by SDS-PAGE using a 12% gel. The molecular mass of the purified proteins was determined using either the Bio-Rad broad range molecular weight standard (M1) (200, 116, 97.4, 66.2, 45.0, 31.0, 21.5, 14.4, 6.5 kDa) or the Amersham Rainbow Marker (M2) (220, 66.2, 46.0, 30.0, 21.5, 14.3 kDa). The molecular weights of GST-fusion proteins are a combination of the 26 kDa GST protein and its fusion partner. Protein concentrations were estimated using the Bradford assay.

**Please replace the paragraph beginning on page 63, line 18, with the following amended paragraph:**

Supernatants for both soluble and insoluble preparations were mixed with 150µl Ni<sup>2+</sup>-resin (previously equilibrated with either buffer A or buffer B, as appropriate) and incubated at room temperature with gentle agitation for 30 min. The resin was CHELATING SEPHAROSE

FAST FLOW(TM) Chelating Sepharose Fast Flow (Pharmacia), prepared according to the manufacturers protocol. The batch-wise preparation was centrifuged at 700xg for 5 min at 4°C and the supernatant discarded. The resin was washed twice (batch-wise) with 10 ml buffer A or B for 10 min, resuspended in 1.0 ml buffer A or B and loaded onto a disposable column. The resin continued to be washed with either (i) buffer A at 4°C or (ii) buffer B at room temperature, the OD<sub>280nm</sub> of the flow-through reached 0.02-0.01. The resin was further washed with either (i) cold buffer C (300mM NaCl, 50mM phosphate buffer, 20mM imidazole, pH 8.0) or (ii) buffer D (8M urea, 10mM Tris-HCl, 100mM phosphate buffer, pH 6.3) until the ~~the~~ OD<sub>280nm</sub> of the flow-through reached 0.02-0.01. The His-fusion protein was eluted by addition of 700µl of either (1) cold elution buffer A (300 mM NaCl, 50mM phosphate buffer, 250mM imidazole, pH 8.0) or (ii) elution buffer B (8 M urea, 10mM Tris-HCl, 100mM phosphate buffer, pH 4.5) and fractions collected until the O.D<sub>280nm</sub> indicated all the recombinant protein was obtained. 20µl aliquots of each elution fraction were analyzed by SDS-PAGE using a 12% gel. Protein concentrations were estimated using the Bradford assay.

**Please replace the two paragraph on page 65, 'ELISA assay (sera analysis)', with the following amended paragraph:**

**ELISA assay (sera analysis)**

The acapsulated MenB M7 strain was plated on chocolate agar plates and incubated overnight at 37°C. Bacterial colonies were collected from the agar plates using a sterile dracon swab and inoculated into 7ml of Mueller-Hinton Broth (Difco) containing 0.25% Glucose. Bacterial growth was monitored every 30 minutes by following OD<sub>620</sub>. The bacteria were let to grow until the OD reached the value of 0.3-0.4. The culture was centrifuged for 10 minutes at 10000 rpm. The supernatant was discarded and bacteria were washed once with PBS, resuspended in PBS containing 0.025% formaldehyde, and incubated for 2 hours at room temperature and then overnight at 4°C with stirring. 100µl bacterial cells were added to each well of a 96 well Greiner plate and incubated overnight at 4°C. The wells were then washed three times with PBT washing buffer (0.1% TWEEN-20(TM) ~~Tween-20~~ in PBS). 200 µl of saturation buffer (2.7%

Polyvinylpyrrolidone 10 in water) was added to each well and the plates incubated for 2 hours at 37°C. Wells were washed three times with PBT. 200 µl of diluted sera (Dilution buffer: 1% BSA, 0.1% TWEEN-20(TM) ~~Tween-20~~, 0.1% NaN<sub>3</sub> in PBS) were added to each well and the plates incubated for 90 minutes at 37°C. Wells were washed three times with PBT. 100 µl of HRP-conjugated rabbit anti-mouse (Dako) serum diluted 1:2000 in dilution buffer were added to each well and the plates were incubated for 90 minutes at 37°C. Wells were washed three times with PBT buffer. 100 µl of substrate buffer for HRP (25 ml of citrate buffer pH5, 10 mg of O-phenildiamine and 10 µl of H<sub>2</sub>O) were added to each well and the plates were left at room temperature for 20 minutes. 100 µl H<sub>2</sub>SO<sub>4</sub> was added to each well and OD<sub>490</sub> was followed. The ELISA was considered positive when OD<sub>490</sub> was 2.5 times the respective pre-immune sera.

Alternatively, The acapsulated MenB M7 strain was plated on chocolate agar plates and incubated overnight at 37°C. Bacterial colonies were collected from the agar plates using a sterile dracon swab and inoculated into Mueller-Hinton Broth (Difco) containing 0.25% Glucose. Bacterial growth was monitored every 30 minutes by following OD<sub>620</sub>. The bacteria were let to grow until the OD reached the value of 0.3-0.4. The culture was centrifuged for 10 minutes at 10 000 rpm. The supernatant was discarded and bacteria were washed once with PBS, resuspended in PBS containing 0.025% formaldehyde, and incubated for 1 hour at 37°C and then overnight at 4°C with stirring. 100µl bacterial cells were added to each well of a 96 well Greiner plate and incubated overnight at 4°C. The wells were then washed three times with PBT washing buffer (0.1% TWEEN-20(TM) ~~Tween-20~~ in PBS). 200 µl of saturation buffer (2.7% Polyvinylpyrrolidone 10 in water) was added to each well and the plates incubated for 2 hours at 37°C. Wells were washed three times with PBT. 200 µl of diluted sera (Dilution buffer: 1% BSA, 0.1% TWEEN-20(TM) ~~Tween-20~~, 0.1% NaN<sub>3</sub> in PBS) were added to each well and the plates incubated for 2 hours at 37°C. Wells were washed three times with PBT. 100 µl of HRP-conjugated rabbit anti-mouse (Dako) serum diluted 1:2000 in dilution buffer were added to each well and the plates were incubated for 90 minutes at 37°C. Wells were washed three times with PBT buffer. 100µl of substrate buffer for HRP (25ml of citrate buffer pH5, 10mg of O-phenildiamine and 10µl of H<sub>2</sub>O<sub>2</sub>) were added to each well and the plates were left at room temperature for 20 minutes. 100µl H<sub>2</sub>SO<sub>4</sub>

was added to each well and OD<sub>490</sub> was followed. The ELISA titers were calculated ~~arbitrarily~~ arbitrarily as the dilution of sera which gave an OD<sub>490</sub> value of 0.4 above the level of preimmune sera. The ELISA was considered positive when the dilution of sera with OD<sub>490</sub> of 0.4 was higher than 1:400.

**Please replace the paragraph on page 68, 'Western Blotting', with the following amended paragraph:**

### **Western Blotting**

Purified proteins (500ng/lane), outer membrane vesicles (5µg) and total cell extracts (25µg) derived from MenB strain 2996 were loaded onto a 12% SDS-polyacrylamide gel and transferred to a nitrocellulose membrane. The transfer was performed for 2 hours at 150mA at 4°C, using transfer buffer (0.3 % Tris base, 1.44 % glycine, 20% (v/v) methanol). The membrane was saturated by overnight incubation at 4°C in saturation buffer (10% skimmed milk, 0.1% ~~Triton~~ TRITON X100(TM) in PBS). The membrane was washed twice with washing buffer (3% skimmed milk, 0.1% ~~Triton~~ TRITON X100(TM) in PBS) and incubated for 2 hours at 37°C with mice sera diluted 1:200 in washing buffer. The membrane was washed twice and incubated for 90 minutes with a 1:2000 dilution of horseradish peroxidase labeled anti-mouse Ig. The membrane was washed twice with 0.1% ~~Triton~~ TRITON X100(TM) in PBS and developed with the OPTI-4CN SUBSTRATE KIT(TM) ~~Opti-4CN Substrate Kit~~ (Bio-Rad). The reaction was stopped by adding water.

**Please replace the paragraph on page 69, 'Gene Variability', with the following amended paragraph:**

### **Gene Variability**

The *ORF4* and *919* genes were amplified by PCR on chromosomal DNA extracted from various *Neisseria* strains (see list of strains). The following oligonucleotides used as PCR primers were designed in the upstream and downstream regions of the genes:

orf 4.1	(forward)	CGAATCCGGACGGCAGGACTC	<u>(SEQ ID NO: 3266)</u>
orf 4.3	(reverse)	GGCAGGGAATGGCGGATTAAAG	<u>(SEQ ID NO: 3267)</u>
919.1	(forward)	AAAATGCCTCTCCACGGCTG or	<u>(SEQ ID NO: 3268)</u>
		CTGCGCCCTGTGTAAATCCCCT	<u>(SEQ ID NO: 3269)</u>
919.6	(reverse)	CAAATAAGAAAGGAATTTTG or	<u>(SEQ ID NO: 3270)</u>
		GGTATCGCAAACTTCGCCTTAATGCG	<u>(SEQ ID NO: 3271)</u>

The PCR cycling conditions were:

1 cycle	2 min. at 94°
30 cycles	30 sec. at 94°
	30 sec. at ~ 54° or ~ 60° (in according to T <sub>m</sub> of the primers)
	40 sec. at 72°
1 cycle	7 min. at 72°

The PCR products were purified from 1 % agarose gel and sequenced using the following primers:

orf 4.1 (forward)	CGAATCCGGACGGCAGGACTC	<u>(SEQ ID NO: 3272)</u>
orf 4.2 (forward)	CGACCGCGCCTTTGGGACTG	<u>(SEQ ID NO: 3273)</u>
orf 4.3 (reverse)	GGCAGGGAATGGCGGATTAAAG	<u>(SEQ ID NO: 3274)</u>
orf 4.4 (reverse)	TCTTTGAGTTTGATCCAACC	<u>(SEQ ID NO: 3275)</u>

**Please replace the paragraph on page 70, line 1, with the following amended paragraph:**

919.1 (forward)	AAAATGCCTCTCCACGGCTG or	<u>(SEQ ID NO: 3276)</u>
	CTGCGCCCTGTGTAAATCCCCT	<u>(SEQ ID NO: 3277)</u>
919.2 (forward)	ATCCTTCCGCCTCGGCTGCG	<u>(SEQ ID NO: 3278)</u>
919.3 (forward)	AAAACAGCGGCACAATCGAC	<u>(SEQ ID NO: 3279)</u>
919.4 (forward)	ATAAGGGCTACCTCAAATC	<u>(SEQ ID NO: 3280)</u>
919.5 (forward)	GCGCGTGGATTATTTTGGG	<u>(SEQ ID NO: 3281)</u>
919.6 (reverse)	CAAATAAGAAAGGAATTTTG or	<u>(SEQ ID NO: 3282)</u>

GGTATCGCAAACTTCGCCTTAATGCG (SEQ ID NO: 3283)

919.7 (reverse)    CCCAAGGTAATGTAGTGCCG    (SEQ ID NO: 3284)

919.8 (reverse)    TAAAAAAAAGTTCGACAGGG    (SEQ ID NO: 3285)

919.9 (reverse)    CCGTCCGCCTGTCGTCGCCC    (SEQ ID NO: 3286)

919.10 (reverse)    TCGTTCCGGCGGGGTCGGGG    (SEQ ID NO: 3287)